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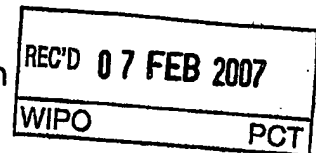
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TRIPLE COMBINATION OF A PYRIMIDINE CONTAINING NNRTI WITH A NUCLEOSIDE RT  
INHIBITOR AND A NUCLEOTIDE RT INHIBITOR

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TRIPLE COMBINATION OF A PYRIMIDINE CONTAINING NNRTI WITH A  
NUCLEOSIDE RT INHIBITOR AND A NUCLEOTIDE RT INHIBITOR

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- 5 The present invention concerns the triple combination of a pyrimidine containing NNRTI with a nucleoside reverse transcriptase inhibitor and a nucleotide reverse transcriptase inhibitor useful for the treatment of HIV infected patients.

BACKGROUND OF THE INVENTION

- 10 Despite the fact that significant progress has been made by the introduction of HAART therapy (Highly Active Anti-Retroviral Therapy), resistance of the HIV virus against nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), nucleotide reverse transcriptase inhibitors (NtRTIs), protease inhibitors and even the more recent fusion inhibitors is still a major cause of therapy failure. For instance, half of the patients receiving anti-HIV combination  
15 therapy do not respond fully to the treatment, mainly because of resistance of the virus to one or more drugs used. Moreover, it has been shown that resistant virus is carried over to newly infected individuals, resulting in severely limited therapy options for these drug-naïve patients. On the International AIDS Conference in Paris in July 2003 researchers released that the biggest study so far of resistance to AIDS drugs finds that  
20 about 10 percent of all newly infected people in Europe have drug-resistant strains. Smaller tests to determine the spread of resistance have been done in the high-risk city center of San Francisco. This test showed the highest level of resistance at 27 percent.

- 25 The pharmacokinetic profile of many commercially available antiretrovirals does not allow relatively low therapeutic doses. Poor pharmacokinetic profiles often in combination with poor solubility properties of the antiretrovirals cause the AIDS patient to face a high pill burden which is particularly undesirable for drug-naïve patients or first line therapy. Moreover, as a consequence of the AIDS virus resisting even antiretroviral combination therapy, a physician will boost the plasma levels of the  
30 active drugs in order for said antiretrovirals to regain effectivity against the mutated HIV viruses. The consequence of which is an even higher increase in pill burden. Boosting plasma levels may also lead to an increased risk of non-compliance with the prescribed therapy and to increased side-effects.

- 35 Several attempts have been made to date to design combination regimens. For instance, the combination of lamivudine (a nucleoside RT inhibitor also named 3TC) at a 150 mg dose and zidovudine (a nucleotide RT inhibitor also named AZT) at a 300 mg dose, formulated in an oral tablet and dosed twice daily, or the combination of abacavir

sulphate at a dose equivalent to 300 mg abacavir (a nucleoside RT inhibitor), lamivudine at a 150 mg dose and zidovudine at a 300 mg dose, formulated in an oral tablet and dosed twice daily.

- 5 WO 93/23021 describes therapeutic combinations for the treatment of HIV-infections comprising zidovudine and an agent serving to enhance the antiviral activity against HIV populations otherwise resistant to zidovudine.

WO 96/01110 describes a triple combination of zidovudine, lamivudine and loviride.

- 10 The latter being a non-nucleoside RT inhibitor of the  $\alpha$ -APA class.

WO 03/016306 specifically discloses more than 250 pyrimidine derivative having HIV replication inhibiting properties that act as non-nucleoside RT inhibitors (NNRTIs) having the ability to inhibit the replication both wild-type and of mutant strains. One of  
15 said NNRTIs is the 4-[[4-[[4-(2-cyanoethenyl)-2,6-dimethylphenyl]-amino]-2-pyrimidinyl]-amino]-benzonitrile (herein named compound A). WO 03/016306 also discloses the methods to synthesize these compounds. It further discloses combinations of said NNRTIs with other antiretrovirals, i.e. suramine, pentamidine, thymopentin, castanospermine, dextran (dextran sulfate), foscarnet-sodium (trisodium phosphono  
20 formate), zidovudine (3'-azido-3'-deoxythymidine, AZT), didanosine (2',3'-dideoxyinosine; ddI), zalcitabine (dideoxycytidine, ddC), lamivudine (2'-3'-dideoxy-3'-thiacytidine, 3TC), stavudine (2',3'-didehydro-3'-deoxythymidine, d4T), abacavir, nevirapine (11-cyclopropyl-5,11-dihydro-4-methyl-6H-dipyrido-[3,2-b : 2',3'-e]  
[1,4]diazepin-6-one), efavirenz, delavirdine, TMC-120, TMC-125, tenofovir,  
25 (S)-8-chloro-4,5,6,7-tetrahydro-5-methyl-6-(3-methyl-2-butenyl)imidazo-[4,5,1-jk][1,4]benzodiazepine-2(1H)-thione,  $\alpha$ -[(2-nitrophenyl)amino]-2,6-dichloro-benzene-acetamide, RO-5-3335, indinavir, ritonavir, saquinavir, lopinavir (ABT-378), nelfinavir, amprenavir, TMC-126, BMS-232632, VX-175, T-20, T-1249, AMD-3100 and hydroxyurea.

30

Notwithstanding existing combination therapy, there is still a need in the art for improved antiretroviral therapy, more particularly AIDS therapy. The need in the art is particularly acute for therapy that is effective not only on wild type HIV virus, but also on the increasingly more common resistant HIV viruses. It is thus highly desirable for  
35 especially first line therapy to design a combination regimen with a low pill burden that limits or even suppresses the recurrence of drug resistant virus and which can be used and remains effective for a long term.

It is an object of the invention to provide a combination of more than one therapeutically effective antiretroviral drug which combination can be used as first line therapy in drug-naïve patients for a long period of time.

- 5 It is also an object of the invention to provide a combination of more than one therapeutically effective antiretroviral drug in which the antiretroviral drugs have a complementary resistance profile thus creating a high resistance barrier and thus allowing a drug-naïve patient to take the combination for a long period of time.
- 10 Another object of the invention is to provide a combination of more than one therapeutically active antiretroviral drug wherein each of the active antiretroviral drugs of the combination can be administered once daily thus reducing the pill burden for the patient.
- 15 A further object of the invention is to provide a combination of more than one therapeutically active antiretroviral drug wherein each of the active antiretroviral drugs of the combination can be co-formulated.

- Yet a further object of the invention is to provide a combination of more than one therapeutically active antiretroviral drug wherein a therapeutically effective amount of each of the active antiretroviral drugs of the combination can be co-formulated in one single pharmaceutical formulation.
- 20

- All references cited herein are incorporated by reference.
- 25

#### DESCRIPTION OF THE INVENTION

- It has been discovered that compound (A) is a potent reverse transcriptase inhibitor that has an extremely high genetic barrier in combination with a favourable pharmacokinetic profile allowing once daily dosing. Based on this discovery, a triple combination is provided comprising (i) compound (A) or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug, and (ii) a nucleoside reverse transcriptase inhibitor, and (iii) a nucleotide reverse transcriptase inhibitor, characterized in that, compound (A) and the nucleotide reverse transcriptase inhibitor and the nucleoside reverse transcriptase inhibitor are therapeutically effective HIV inhibitors at a dose that can be administered once daily.
- 30
- 35

It was surprising to discover that compound (A) has all these properties together. This is unusual because one cannot predict what mutations will be selected in the HIV-1 genome by a given drug, whether the mutated virus will have any chance of survival

under the pressure of the drug, how much drug is needed to limit or to suppress the recurrence of such mutated virus, and at what frequency such drug has to be given to maintain suppression of the development of a resistant virus that can break through the genetic barrier of the drug.

5

Advantageously, the nucleotide reverse transcriptase inhibitor and the nucleoside reverse transcriptase inhibitor select mutations in the reverse transcriptase that do not cause resistance to compound (A). Therefore, in a preferred embodiment, a triple combination is provided comprising (i) compound (A) or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug, and (ii) a nucleoside reverse transcriptase inhibitor, and (iii) a nucleotide reverse transcriptase inhibitor, characterized in that, (1) compound (A) and the nucleotide reverse transcriptase inhibitor and the nucleoside reverse transcriptase inhibitor are therapeutically effective HIV inhibitors at a dose that can be administered once daily and (2) the nucleotide reverse transcriptase inhibitor and the nucleoside reverse transcriptase inhibitor select mutations in the reverse transcriptase that do not cause resistance to compound (A).

Compound (A) can also be used in a method for treating HIV infected patients that includes administering compound (A) or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug, in combination with a nucleoside reverse transcriptase inhibitor, and a nucleotide reverse transcriptase inhibitor, in which method a therapeutically effective amount of compound (A), the nucleotide reverse transcriptase inhibitor and the nucleoside reverse transcriptase inhibitor can be administered once daily. One embodiment of the present invention provides for the present combination for use as a medicine. In another embodiment, the combination of the present invention can be used in the manufacture of a medicament to treat HIV infected patients.

The present triple combination is especially useful for the treatment of AIDS and related clinical conditions such as AIDS related complex (ARC), progressive generalised lymphadenopathy (PGL) or AIDS related neurological conditions such as multiple sclerosis. The present triple combination may be particularly useful for the treatment of drug-naïve HIV infected patients.

In a preferred embodiment, each of the ingredients of the present triple combination can be co-formulated in one pharmaceutical form and do not have to be administered in a separate pharmaceutical form. The daily therapeutic antiretroviral amount of the ingredients of the present triple combination of such co-formulated single pharmaceutical form may then be given in a single unit dosage form or even in multiple

unit dosage forms, such as two, three, four, five or even more unit dosage forms. A physician will be able to determine the exact dosage to be given taking into account the severity of the patient's condition as well as the patient's weight, gender and possibly other parameters such as individual differences in absorption, biodistribution, metabolism and excretion rates for each drug as well as other factors known to those skilled in the art.

Thus, in one embodiment, a pharmaceutical composition is provided comprising a pharmaceutically acceptable carrier and as active ingredients (i) compound (A) or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug, and (ii) a nucleoside reverse transcriptase inhibitor, and (iii) a nucleotide reverse transcriptase inhibitor.

Preferred nucleotide reverse transcriptase inhibitors that can be used in the present triple combination and methods employing such triple combination include tenofovir and its prodrug tenofovir disoproxil fumarate.

Tenofovir is an adenosine nucleotide analogue currently commercially available with activity against retroviruses. Tenofovir disoproxil fumarate (tenofovir DF) is a once-daily, orally administered prodrug of the intravenously administered antiviral agent tenofovir (PMPA). For antiviral activity, tenofovir DF needs to be hydrolysed to the ANP analogue and then phosphorylated to the active diphosphate moiety [Arimilli et al. *Antiviral Chemistry and Chemotherapy* 1997, 8 :6 (557-564); Fridland et al. *Antiviral Research* 1997, 34]. After entry in to lymphocytes or macrophages, the prodrug is quantitatively converted to the parent analogue, tenofovir, and phosphorylated to mono- and diphosphate metabolites. The cellular enzymes that are responsible for phosphorylation of this drug are adenylate kinase and nucleoside diphosphate kinase [Robbins et al. *Antimicrobial Agents and Chemotherapy* 1995, 39 :10 (2304-2308); Robbins et al. *Antimicrobial Agents and Chemotherapy* 1998, 42 :3 (612-617)]. Unlike other nucleoside analogues, such as zidovudine or stavudine, both of whose phosphorylation is cell cycle-dependent, tenofovir is efficiently phosphorylated in resting as well as cycling peripheral blood lymphocytes [Robbins et al. 1998]. Tenofovir can inhibit HIV-1 replication in different cell types that may target HIV, including primary human blood lymphocytes and macrophages [Perno et al. *Antiviral Research* 1992 (289-304); Perno et al. *Molecular Pharmacology* 1996, 50 :2 (359-366)]. The primary target of tenofovir diphosphate is reverse transcriptase (RT). Tenofovir diphosphate is a competitive inhibitor for the incorporation of deoxyadenosine triphosphate into nascent proviral DNA chains. Inhibition of HIV-1



RT by tenofovir diphosphate has an inhibition constant of approximately 0.9  $\mu\text{M}$ , and if the analogue is incorporated into the growing viral DNA chain it may terminate further chain elongation. Tenofovir inhibits viral RT much more effectively than it inhibits cellular DNA polymerases [Suo et al *Journal of Biological Chemistry* 1998, 273 :42 (2750-2758)]. The concentration required to inhibit the replication of various HIV-1 strains by 50% (EC50) in lymphocyte and macrophage cell types (MT-2, CEM, ACH8) ranges from 0.2 to 10  $\mu\text{M}$ . The antiviral effect is achieved at non-toxic doses of tenofovir (selectivity index ranging from 100 to 2000). Tenofovir DF is currently available as 300 mg tablets to be taken once daily.

10 Viral resistance to tenofovir in vitro emerges slowly. A recombinant virus expressing the K65R mutation showed a 3-fold decreased susceptibility to tenofovir in vitro [Cherrington et al. *Interscience Conference on Antimicrobial Agents and Chemotherapy* 1997, 37th]. Notably, clinical HIV strains expressing the M184V  
15 lamivudine-associated resistance mutation on RT show wild-type or increased susceptibility to tenofovir in vitro, independent of changes in  $K_i$  for the mutant enzyme [Miller et al. *Interscience Conference on Antimicrobial Agents and Chemotherapy* 1998,]. Long-term treatment (5 to 15 weeks) of newborn rhesus macaques with tenofovir (doses of 30 mg/kg) starting 3 weeks after inoculation with simian  
20 immunodeficiency virus, resulted in emergence of SIV with approximately 5-fold decreased susceptibility to tenofovir [van Rompay et al. *Antimicrobial Agents and Chemotherapy* 1996, 40 :11 (2586-2591)]. This low level of resistance was associated with the appearance of the K65R mutation.

25 In a preferred embodiment, a triple combination is provided comprising (i) compound (A) or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug, and (ii) a nucleoside reverse transcriptase inhibitor, and (iii) tenofovir or its prodrug tenofovir disoproxil fumarate, characterized in that, compound (A) and the nucleoside reverse transcriptase inhibitor and tenofovir or its prodrug tenofovir disoproxil  
30 fumarate are therapeutically effective HIV inhibitors at a dose that can be administered once daily.

In a preferred embodiment, a triple combination is provided comprising (i) compound (A) or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug, and  
35 (ii) a nucleoside reverse transcriptase inhibitor, and (iii) tenofovir disoproxil fumarate, characterized in that, compound (A) and the nucleoside reverse transcriptase inhibitor and tenofovir disoproxil fumarate are therapeutically effective HIV inhibitors at a dose that can be administered once daily.

Preferred nucleoside reverse transcriptase inhibitors that can be used in the present triple combination and methods employing such triple combination include emtricitabine, racemic FTC and lamivudine (also named 3TC).

5 Emtricitabine or (-)-FTC is the left (-) rotatory enantiomeric form of racemic FTC or ( $\pm$ )-cis-4-amino-5-fluoro-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-2(1H)-pyrimidinone (FTC). It is a commercially available nucleoside analogue and exhibits activity against HIV-1 [Hoong et al. *Journal of Organic Chemistry* 1992 (5563-5565);  
10 Jeong et al *Journal of Medicinal Chemistry* 1993, 36 :2 (181-195); Van Roey et al. *Antiviral Chemistry and Chemotherapy* 1993, 4 :6 (369-375]. The in vitro anti-HIV-1 activity of (-)-beta-enantiomer of FTC was reported to be 20-fold more than the (+)-beta-enantiomer, and the (+)-enantiomer was significantly more toxic than the (-)-enantiomer to myeloid progenitor cells [Schinazi et al *Antimicrobial Agents and*  
15 *Chemotherapy* 1992, 36 :11 (2423-2431)]. The potential for HIV-1 resistance to FTC was evaluated by serial passage of the virus in human PBMCs and MT-2 cells in the presence of increasing drug concentrations. Highly drug-resistant HIV-1 variants dominated the replicating virus population after two or more cycles of infection. RT derived from drug-resistant viral particles was 15- to 50-fold less susceptible to the  
20 5'-triphosphate of FTC compared with the enzyme from parental drug susceptible virus. DNA sequence analysis of the RT gene amplified from resistant viruses consistently identified mutations at codon 184 from Met (ATG) to Val (GTG or GTA) [Schinazi et al *Antimicrobial Agents and Chemotherapy* 1993, 37 :4 (875-881); Tisdale et al *Antiviral Research* 1993, 20 :Suppl 1; Smith et al *Journal of Virology*  
25 1997, 71 :3 (2357-2362); Harrer et al *Journal of Infectious Diseases* 1996, 173 :2 (476-479); Tisdale et al *Proceedings of the National Academy of Sciences of the United States of America* 1993, 90 :12 (5653-5656)]. Due to this observed single mutation in the YMDD of reverse transcriptase in the HIV-infected patients, (-)-FTC is not suitable for monotherapy and needs to be administered in combination with other  
30 antiretroviral agents to effectively treat patients infected with HIV. Emtricitabine is available as 200 mg capsules to be taken once a day.

Lamivudine has the chemical name (-)-2',3'-dideoxy-3'-thiacytidine and is described for instance in EP 382,526 as an antiviral nucleoside analogue. It is also a well  
35 established and useful antiretroviral which is commercially available for instance as 150 mg oral tablets. Lamivudine is also commercially available in combination with zidovudine (300 mg zidovudine / 150 mg lamivudine), and in combination with

lamivudine and abacavir sulphate (300 mg zidovudine / 150 mg lamivudine / equivalent of 300 mg abacavir).

5 In a preferred embodiment, a triple combination is provided comprising (i) compound (A) or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug, and (ii) emtricitabine, and (iii) a nucleotide reverse transcriptase inhibitor, characterized in that, compound (A) and the nucleotide reverse transcriptase inhibitor and emtricitabine are therapeutically effective HIV inhibitors at a dose that can be administered once daily.

10

In another preferred embodiment, a triple combination is provided comprising (i) compound (A) or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug, and (ii) lamivudine, and (iii) a nucleotide reverse transcriptase inhibitor, characterized in that, compound (A) and the nucleotide reverse transcriptase inhibitor and lamivudine are therapeutically effective HIV inhibitors at a dose that can be administered once daily.

15

In another preferred embodiment, a triple combination is provided comprising (i) compound (A) or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug, and (ii) emtricitabine, and (iii) tenofovir or its prodrug tenofovir disoproxil fumarate, characterized in that, compound (A) and emtricitabine and tenofovir or its prodrug tenofovir disoproxil fumarate are therapeutically effective HIV inhibitors at a dose that can be administered once daily.

20

25 The following preferred triple combinations are also included

(a) compound (A) or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug with emtricitabine and tenofovir disoproxil fumarate;

(b) compound (A) or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug with lamivudine and tenofovir disoproxil fumarate.

30

A preferred form of compound (A) is the E-isomer, i.e. (E)- 4-[[4-[[4-(2-cyanoethenyl)-2,6-dimethylphenyl]-amino]-2-pyrimidinyl]-amino]-benzonitrile (hereinafter called compound E-(A)). The Z-isomer of compound (A) (hereinafter called compound Z-(A)) has relatively high potency against wild-type HIV-1, and also finds use in the present triple combinations. However, it is less active against single and double mutants in comparison to the E-isomer. Table 1 shows the IC<sub>50</sub> value in nM of the E and Z-isomer of compound A.

35

**Table 1**

HIV RT mutation	E-isomer	Z-isomer
Wild-type	0.4	0.6
100I	0.4	6.3
103N	0.3	1.6
181C	1.3	5.0
188L	2.0	32
227C	2.0	4.0
100I+103N	7.9	790
103N+181C	1.0	40
227L+106A	1.0	4.0

The respective daily dose for each of the compounds of the present combination may range between 10 mg and 800 mg, preferably between 50 and 400 mg, more preferably  
5 between 100 and 300 mg.

The weight ratio of each couple of components of the triple combination taken on a daily basis may vary in a range from 1/10 to 10/1. Suitably, the weight ratio of each couple varies between 1/4 and 4/1, preferably between 1/3 and 3/1, and more preferably  
10 between 1/2 and 2/1.

The compounds of the present triple combination may be administered simultaneously, concurrently or sequentially. Simultaneous administration may be done by employing a unitary pharmaceutical formulation or separate pharmaceutical formulations. In  
15 general, the combination may be administered by topical, oral, rectal, intravenous, subcutaneous or intramuscular routes. For first line therapy of HIV infection, simultaneous administration employing a unitary pharmaceutical formulation is preferred.

## 20 **EXPERIMENTAL PART**

### Pharmacokinetics of compound E-(A)

A double-blind, randomized, placebo-controlled Phase I trial was designed to evaluate safety, tolerability, and *ex-vivo* pharmacokinetics of single doses of compound E-(A) in healthy male volunteers. Oral doses of 12.5, 25, and 50 mg were formulated in PEG 400 and taken with a standard meal. The pharmacokinetic results are shown in Table 2.

The pharmacokinetic results of another double-blind, randomized, placebo-controlled Phase I study with 4 dosing sessions to evaluate the safety, tolerability, pharmacokinetics and *ex-vivo* pharmacodynamics of single 100 mg and 200 mg oral

doses of compound E-(A) in healthy male subjects are also reported in Table 2. Randomization was such that for each session 6 subjects received the same dose of compound E-(A) and 3 subjects received placebo. There was a time interval of about 14 days between each dosing session

Table 2 shows that high and dose-proportional exposures were obtained. The correlation coefficient for the 5  $C_{max}$  datapoints is 0.9897 and for the area under the curve values between 0 and 48 hours ( $AUC_{0-48hr}$ ) 0.9952. Half-life of plasma concentrations ranged between 37 and 39 hours. The compound was well tolerated by the volunteers. No relevant adverse effects of the drug were noted.

Table 2

Parameter	12.5 mg	25 mg	50 mg	100 mg	200 mg
$C_{max}$ (ng/ml)	73±14	149±32	267±27	482±121	807 ±207
$T_{max}$ (hr)	4.0±0	4.0±1.3	4.0±1.3	4.3±0.8	4.3 ±0.8
$AUC_{0-48hr}$ (nghr/ml)	1337±310	2805±496	5094±509	8162±2251	15592±2746
$AUC_{0-\infty}$ (nghr/ml)	2210±473	4637±1164	8872±1342	15844±4592	
$T_{1/2}$ (hr)	37.1	38.7	45±9	55±18	

#### Virological profile of Compound E-(A)

- 5 Compound E-(A) was tested in a cell-based assay, using natural host cells of HIV. MT-4 cells (a cell line of human T cells) were infected with HIV-1 (wild type or mutants) and exposed to different concentrations of antiviral compound in the presence of 10% fetal calf serum. Cytotoxicity was determined in parallel with the antiviral activity so that the selectivity of the antiviral effect could be assessed. Active compounds have to
- 10 penetrate the cell membrane in order to interfere with replication steps inside the cell. After four days of incubation at 37°C, the viability of the HIV and mock-infected cells was assessed by an automated tetrazolium-based colorimetric assay. This method enabled the calculation of both the 50% inhibitory concentration for inhibition of viral cytopathicity (IC<sub>50</sub>), the IC<sub>90</sub>, and the 50% cytotoxic concentration (CC<sub>50</sub>). The ratio
- 15 CC<sub>50</sub>/IC<sub>50</sub>, also called the selectivity index, is an indication of the specificity of the antiviral effect. Tested HIV strains included: Wild type (wt) HIV-1; a panel of single and double mutants, obtained by site-directed mutagenesis (SDM), and a panel of clinical isolates, selected for resistance against NNRTIs.
- 20 *Activity towards wild type and SDM mutants*  
A limited panel of HIV-1 mutants was constructed using site-directed mutagenesis

(SDM) and homologous recombination techniques. Compound E-(A) was tested against an extended panel of single and double mutants known to be resistant against commercially available NNRTIs. Nevirapine (NVP) and efavirenz (EFV) were included as controls.

5

The results are shown in Table 3 (values presented are IC<sub>50</sub> values in nM). For wild type virus, the obtained IC<sub>50</sub> was 0.4 nM (0.15 ng/ml) and the IC<sub>90</sub> 1.3 nM (0.48 ng/ml). The HIV strain with the lowest sensitivity against compound E-(A) within this selection was the double mutant 100I+103N, with an IC<sub>50</sub> of about 8 nM and an IC<sub>90</sub> of about 16 nM.

10

Table 3

	NVP	EFV	Compound E-(A)
wild type	81	1.4	0.4
100I	597	35	0.4
101E	547	5	1.6
103N	2,879	28	0.3
106A	2,983	23	0.2
108I	-	2	0.3
138K	64	1.3	0.4
179D	161	6	0.6
179E	158	5	0.4
181C	10,000	2	1.3
188C	3,764	5	0.1
188H	241	9	0.2
188L	10,000	78	2.0
190A	4,101	8	0.3
190S	10,000	275	0.1
225H	171	2	0.3
227C	1,816	36	2.0
227L	78	0.3	0.3
234I	45	NT	0.3
236L	41	1	0.3
100I+103N	10,000	10,000	7.9
101E+103N	7,033	84	0.5
103N+181I	10,000	37	1.0
227L+106A	10,000	8	1.0

*Development of resistance in vitro*

NNRTIs are highly selective inhibitors of HIV-1 but their current clinical use is limited by the rapid emergence of NNRTI (cross-) resistance. The rate of resistance emergence against compound E-(A) and the first generation NNRTIs nevirapine and efavirenz was compared in vitro.

MT4 cells were infected with wild type HIV-1 at high multiplicity of infection ( $>1$  infectious virus per cell, to maximize the genetic diversity of the virus population) in the presence of various concentrations of compound E-(A) (40, 200, 1000 and 5000  $\times$  IC<sub>50</sub>), and were monitored twice a week for virus replication. Emerging virus was collected for pheno- and genotyping. Cultures without evidence of virus replication were further sub-cultivated in the presence of the same concentration of inhibitor for a total duration of 30 days (10 passages).

Resistance to nevirapine emerged within 3-6 days, at all tested concentrations. Breakthrough virus harboured the typical Y181C mutation. The same experiments with efavirenz resulted in the selection of G190E at all concentrations (up to 5  $\mu$ M) within 3 to 7 days. Compound E-(A) did not select for resistant virus within 30 days using wild-type virus. If a double resistant mutant K103N+Y181C (IC<sub>50</sub> 0.8 nM) was used instead of wild type virus, resistance did emerge at all tested concentrations. Starting from the single mutants Y181C (IC<sub>50</sub> 1.3nM) or 103N (IC<sub>50</sub> 0.3nM), virus breakthrough did not occur at 40 and 200 nM, but did occur at 10 nM.

In this experimental setting of high genetic diversity, HIV-1, resistant to first generation NNRTIs, was selected very rapidly. Resistant viruses harboured only one mutation. In contrast, emergence of HIV-1, resistant to compound E-(A) was delayed or did not occur.

Cardiovascular and pulmonary safety of compound E-(A)

Compound E-(A) had little or no effect on cardiovascular and pulmonary parameters in vivo at plasma levels covering and exceeding the targeted plasma levels in man and at concentrations in vitro covering or exceeding the anti-viral concentration in vitro.

## CLAIMS

1. A combination comprising
  - (i) 4-[[4-[[4-(2-cyanoethenyl)-2,6-dimethylphenyl]-amino]-2-pyrimidinyl]-amino]-benzonitrile, also named compound (A), or a stereoisomeric form or a  
5 pharmaceutically acceptable salt or a prodrug thereof, and
  - (ii) a nucleoside reverse transcriptase inhibitor, and
  - (iii) a nucleotide reverse transcriptase inhibitor,  
characterized in that, compound (A) and the nucleotide reverse transcriptase  
inhibitor and the nucleoside reverse transcriptase inhibitor are therapeutically  
10 effective HIV inhibitors at a dose that can be administered once daily.
2. A combination according to claim 1 wherein compound (A) occurs in its  
E-isomeric form.
3. A combination according to claims 1 or 2 wherein the nucleotide reverse  
transcriptase inhibitor and the nucleoside reverse transcriptase inhibitor select  
15 mutations in the reverse transcriptase that do not cause resistance to compound (A).
4. A combination according to any one of claims 1 to 3 wherein the nucleotide reverse  
transcriptase inhibitor is tenofovir or its prodrug tenofovir disoproxil fumarate.
5. A combination according to any one of claims 1 to 4 wherein the nucleoside reverse  
transcriptase inhibitor is emtricitabine, racemic FTC or lamivudine (also named  
20 3TC).
6. A combination according to any one of claims 1 to 5 wherein the nucleoside reverse  
transcriptase inhibitor is emtricitabine.
7. A combination according to any one of claims 1 to 6 wherein the combination  
comprises
  - (i) E-4-[[4-[[4-(2-cyanoethenyl)-2,6-dimethylphenyl]-amino]-2-pyrimidinyl]-  
25 amino]-benzonitrile or a pharmaceutically acceptable salt, and
  - (ii) tenofovir or its prodrug tenofovir disoproxil fumarate, and
  - (iii) emtricitabine.
8. A combination according to any one of claims 1 to 5 wherein the nucleoside reverse  
30 transcriptase inhibitor is lamivudine.
9. A combination according to claim 8 wherein the combination comprises
  - (i) E-4-[[4-[[4-(2-cyanoethenyl)-2,6-dimethylphenyl]-amino]-2-pyrimidinyl]-



amino]-benzonitrile or a pharmaceutically acceptable salt, and  
(ii) tenofovir or its prodrug tenofovir disoproxil fumarate, and  
(iii) lamivudine.

- 5 10. A combination according to any one of claims 1 to 9 wherein weight ratio of each couple of components of the triple combination taken on a daily basis may vary in a range from 1/4 to 4/1.
- 10 11. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and as active ingredients (i) compound (A) or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug, and (ii) a nucleoside reverse transcriptase inhibitor, and (iii) a nucleotide reverse transcriptase inhibitor.
12. A combination as claimed in any one of claims 1 to 10 for use as a medicine.

ABSTRACT

TRIPLE COMBINATION OF A PYRIMIDINE CONTAINING NNRTI WITH A  
NUCLEOSIDE RT INHIBITOR AND A NUCLEOTIDE RT INHIBITOR

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The present invention concerns the triple combination of a pyrimidine containing  
NNRTI with a nucleoside reverse transcriptase inhibitor and a nucleotide reverse  
transcriptase inhibitor useful for the treatment of HIV infected patients. It further  
10 relates to pharmaceutical formulations containing such triple combination.